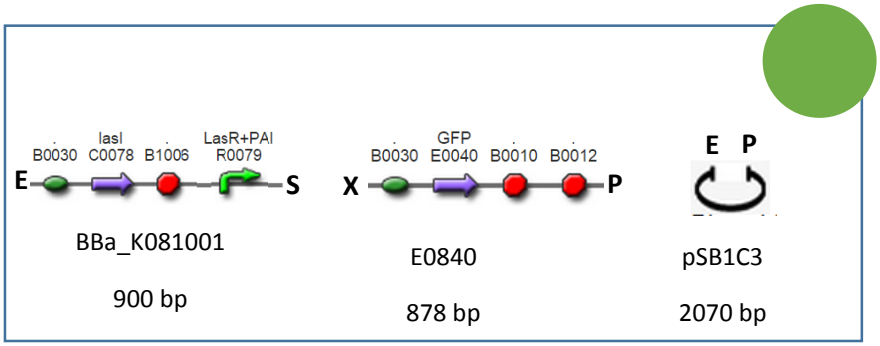
Assembly:

A III



1st Day

EXSP Digestion (see **Enzymatic Digestion Protocol**)

Parts	ng/ul	Volume to 2,5 ug (ul)	Buffer x10 (ul)	EcoRI (ul)	XbaI (ul)	SpeI (ul)	PstI (ul)	H_2O to 50ul (ul)
BBa_K081001	171,3	14,6	5	1	-	1	-	28,4
BBa_E0840	156,8	15,9	5	-	1	-	1	27,1
pSB1C3	107,3	23,3	5	1	-	-	1	20,7

2nd Day

Gel Purification

- See **Kit Wizard SV gel and PCR clean up Promega Protocol**
- Quantify digestion products

Parts	ng/ul	260/280
BBa_K081001 (ES)	16,0	1,65
BBa_E0840 (XP)	16,6	1,79
pSB1C3 (EP)	24,3	2,83

Obs: 260/280 in a quality parameter that tells you if your sample is contaminated with proteins. The greater it is compared to 1 the less contaminants you have.

Ligation (see **Ligation Protocol**)

Linear Plasmid 50 ng	2 ul	
Insert : Plasmid 3:1 (both)	BBa_K081001	BBa_E0840
	4 ul	4 ul
10x T4 DNA Buffer	2 ul	
T4 DNA ligase 1u	1 ul	
H_20 to 20 ul	7 ul	

Obs: To determinate the amount of DNA necessary we used the following equation

$$Insert\ ng = plasmid\ ng \times \frac{insert\ bp}{plasmid\ bp} \times insert{:}\,plasmid\ ratio$$

- Incubate overnight at 37°C.
- Prepare and sterilize in the autoclave tubes with 6 ml of liquid LB medium
- Prepare glycerol 40%

3rd Day

Transformation (see **Transformation Protocol in *Escherichia coli* DH5-α**)

Organism: DH5 - α

Selection: Cloranphenicol

4th Day

- Inoculate 3 – 4 colonies in a 6 ml LB with the same antibiotic used in the transformation protocol.
- Incubate overnight at 275rpm/37°C.

5th Day

Miniprep

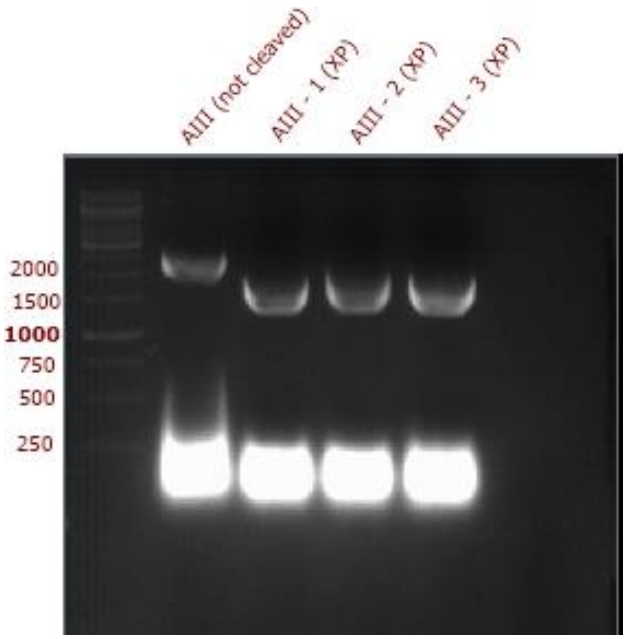
- Prepare **glycerol stock** of the clones (500ul glycerol 40% + 500ul inoculum).
- Extract plasmidial DNA (see **Alkaline Lyses or PureLink Invitrogen Protocol**)
- Run a preliminary electrophoresis gel.
- Quantify DNA samples.

Assembly Confirmation

- XP Digestion (see **Enzymatic Digestion Protocol**)

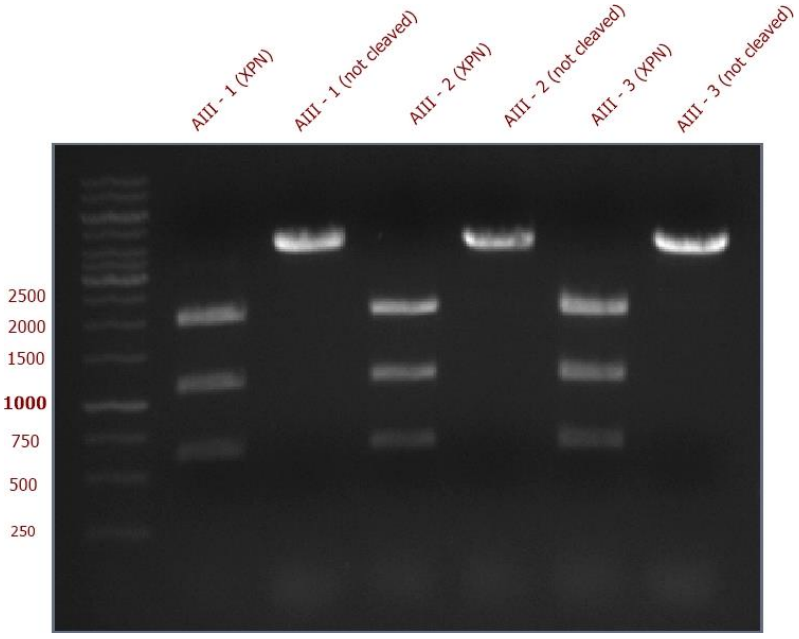
Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	XbaI (ul)	PstI (ul)	H_2O to 10ul (ul)
AIII – 1	3	1	0,5	0,5	5
AIII – 2	3	1	0,5	0,5	5
AIII – 3	3	1	0,5	0,5	5

- Incubate for 2 hours at 37°C.
- Prepare samples for DNA sequencing.
- Run an electrophoresis analysis of the XP digestion



Obs: Here we had a problem with the length of the DNAs. Our assembly has around 1800 bp, while the pSB1C3 has 2070 bp. Because of this, the bands were not able to run apart as we wanted them to. As a solution to this problem, we performed a triple digestion of the AIII clones using XbaI, PstI and NdeI. The last one cleaves specifically the AIII assembly in the BBa_E0840. As a result of this digestion we expected three different bands: a 625 bp from the BBa_E0840, a 1200 bp from part of the BBa_E0840 plus the BBa_K081001 and the 2070 bp pSB1C3.

Assembly	Volume to 300 ng (ul)	Buffer x10 (ul)	XbaI (ul)	NdeI (ul)	PstI (ul)	H_2O to 10ul (ul)
AIII – 1	3	1	0,5	0,5	0,5	4,5
AIII – 2	3	1	0,5	0,5	0,5	4,5
AIII – 3	3	1	0,5	0,5	0,5	4,5



Size expected	Size in gel
1778 bp	~ 1900 bp